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Article title	Characterisation of recombinant isoforms of birch pollen allergen Bet v 1
Article identifier	0065259897014826
Authors	Spangfort_M_D Ipsen_H Sparholt_S_H Aasmul-Olsen_S Osmark_P Poulsen_F_M Larsen_M Mortz_E Roepstorff_P Larsen_J_N
Journal title	Advances in Experimental Medicine and Biology
ISSN	0065-2598
Publisher	Plenum
Year of publication	1996
Volume	409
Issue	1996
Supplement	0
Page range	251-254
Number of pages	4
User name	Adonis
Cost centre	Development
PCC	\$16.00
Date and time	Monday, February 12, 2001 9:31:11 AM

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## CHARACTERISATION OF RECOMBINANT ISOFORMS OF BIRCH POLLEN ALLERGEN Bet v 1

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### 1. ABSTRACT

Three isoforms of the major birch pollen allergen, Bet v 1 from *Betula verrucosa* have been expressed as recombinant proteins in *E. coli* and purified. The immunochemical properties of recombinant isoforms (rBet v 1) differed on immunoblots when compared using Mabs and birch pollen allergic patients serum IgE. 2-D gel analysis showed that recombinant isoforms with different epitope structure can focus under the same protein spot after electrophoresis. The structure of conformational epitopes can be distorted by amino acid substitutions even when T-cell epitopes are not affected as judged by T-cell proliferation studies.

### 2. INTRODUCTION

The major birch pollen allergen Bet v 1 is a protein with an apparent molecular weight of 17 kDa. About 20 different isoforms of Bet v 1 can be identified in birch pollen extract by 2-D immunoblotting using monospecific antibodies [1,2]. Using Mabs or sera from individual birch pollen allergic patients, differences in individual reactivity towards different isoforms suggest differences in epitope structure [1]. These observations are in agreement with a model in which differences in amino acid sequence induces differences in epitope structure. However, a detailed characterisation of the epitope structure and molecular properties of Bet v 1 is lacking due to difficulties of isolating individual Bet v 1 isoforms from pollen extract. In this work, we have addressed this problem by producing

	010	020	030	040	050	060	070	080
2230:	GVPNYETETT	SVIPAARLFF	AFILDGDNLF	PKVAPQAIS	VENIEGNGGP	GTIKKISFPE	GFPFKYVKDR	VDEVDHTNFK
2226:	.....	.....	.....	.....	.....	.....G	.....L	.....
2227:	.....	.....	.....	.....	.....	.....L	.....	.....
	090	100	110	120	130	140	150	
2230:	YNYSVIEGGP	IGDTLEKISN	EIKIVATPDG	GSILKISNKY	HTKGDHEVKA	EQVKASKEMG	ETLLRAVESY	LLAHSDAYN
2226:	.....	.....	.....CV	.....N	.....	.....	.....	.....
2227:	.....	.....	.....	.....	.....	.....	.....	.....

Figure 1. Isoforms of Bet v 1 expressed as recombinant proteins in *E. coli*.

purified Bet v 1 isoforms as recombinant proteins in order to compare their immunochemical and molecular properties.

### 3. MATERIAL AND METHODS

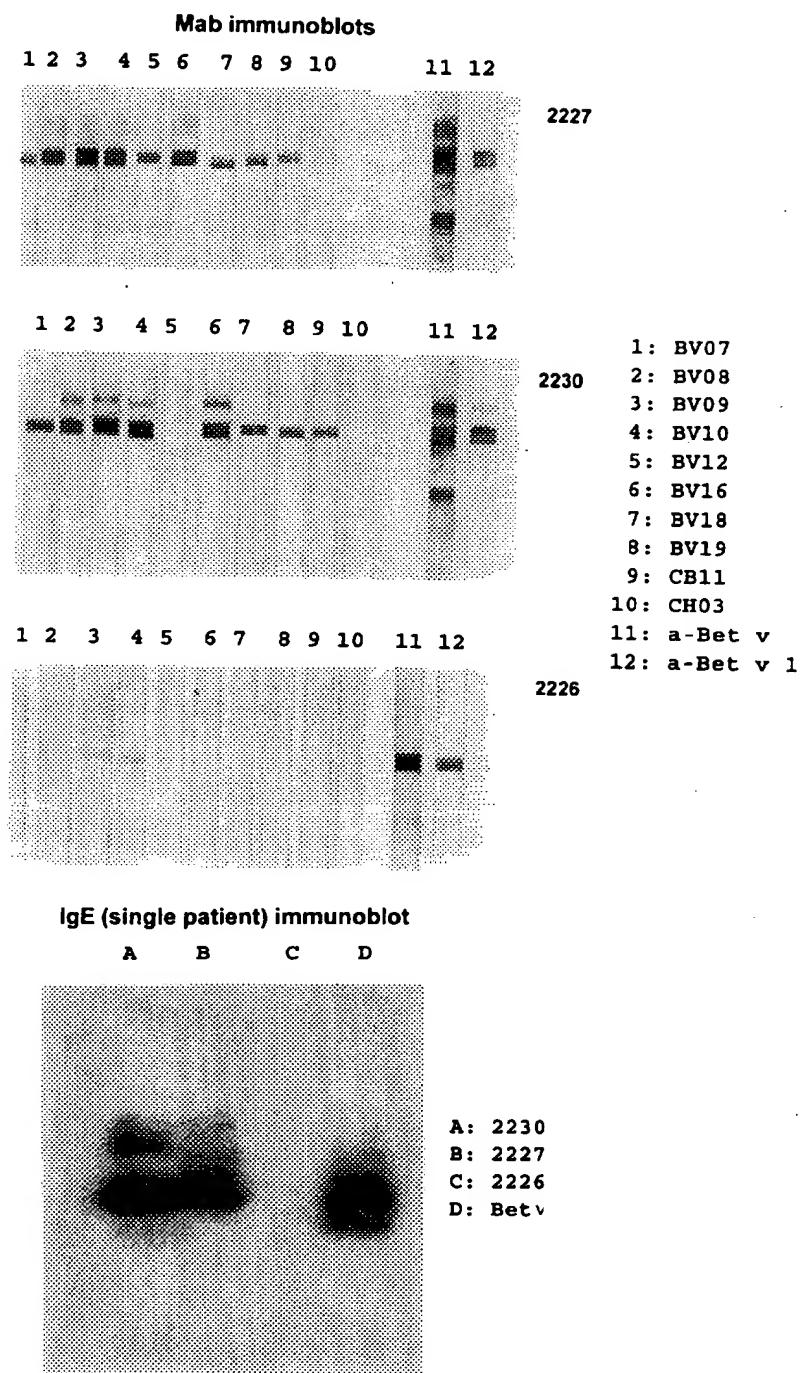
RNA was purified from *Betula verrucosa* pollen and genes encoding Bet v 1 were specifically amplified by PCR as in [3]. The products were subcloned into the maltose-binding protein fusion vector pMAL-c and expressed in *E. coli*. Affinity purified fusion protein was enzymatically cleaved into its two protein constituents by incubation with Factor Xa, followed by gel filtration to isolate rBet v 1.

### 4. RESULTS AND DISCUSSION

The primary amino acid sequences of the rBet v 1 isoforms 2230, 2227 and 2226 as deduced from their respective genes are shown in Fig. 1. The purified isoforms were characterised by SDS-PAGE, analytical gelfiltration, mass spectrometry, N-terminal sequencing and NMR spectroscopy.

#### 4.1. Immunological Characterisation

Fig. 2 show that isoform 2227 is recognised by all Mabs tested, whereas isoform 2230 does not bind Mab BV12. Isoform 2227 and 2230 differ by a hydrophobic amino acid substitution, Phe to Leu, which apparently abolish BV12 binding. The reactivity of isoform 2226 on immunoblots was generally weaker compared to other isoforms. It did however, react readily with a polyclonal rabbit anti-Bet v 1 antibody. Recombinant isoforms were also tested on immunoblots against a pool of birch pollen allergic patients serum IgE. Isoforms 2227 and 2230 strongly bound patients IgE, whereas no IgE-binding to 2226 could be detected. This demonstrates that relatively small changes in the amino acid composition can have large effects on the structure of conformational epitopes. The antigenic activity of recombinant Bet v 1 isoforms was further characterised by their ability to induce T-cell proliferation in a longterm Bet v 1 reactive T-cell line (not shown). All three isoforms elicited a strong proliferation response in rates comparable to naturally occurring Bet v 1.



**Figure 2.** Immunoblotting of purified recombinant Bet v 1 against a panel of Mabs (left) and against birch pollen allergic patients serum IgE (right).

#### 4.2. 2-D Gel Electrophoresis of Bet v 1 Isoforms and Birch Extract

When analysed by 2-D electrophoresis (not shown), isoforms 2230 and 2227 gave identical 2-D profiles which demonstrates that different isoforms can be located under the same protein spot on a 2-D gel. Since isoform 2227 but not 2230 reacts with Mab BV12, it also demonstrates that isoforms with different epitope structure can be present under the same 2-D gel protein spot. Thus, the resolution power of the 2-D gel system is limited as it cannot account for the full spectrum of immunochemical and sequence heterogeneity of Bet v 1. Apart from a major protein spot, all isoforms gave rise to up to three additional minor spots which probably represents artefacts generated by the 2-D gel system.

In summary, although these data suggests that even single amino acid substitutions apparently affects epitope structure, the reported immunochemical characterisation is limited to immunoblots only. In theory, the lack of reactivity against Mabs and/or serum IgE could be accounted for by incorrect re-folding of the protein after SDS-PAGE. Further characterisation using fluid-phase inhibition assays are in progress.

#### 5. REFERENCES

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2. Jarolim, A., Rumpold, H., Endler, T., Ebner, H., Breitenbach, M., Scheiner, O. and D. Kraft (1989) *Allergy* 44: 385-395.
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